A Scientific Review of the Potential Environmental Effects of Aquaculture in Aquatic Ecosystems

Volume II

Disease Interactions Between Wild and Cultured Shellfish
(S. M. Bower and S. E. McGladdery)

Fisheries and Oceans Canada
Science Sector
200 Kent Street
Ottawa, Ontario K1A 0E6
Canada

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TABLE OF CONTENTS

Editorial Board iv
Foreword v
Avant-propos vii

Disease Interactions Between Wild and Cultured Shellfish
(S.M. Bower and S.E. McGladdery)

Executive Summary 1
Résumé 4
Background 8
'New Diseases' 9
Opportunistic Infections 10
'Exotic' or Non-Indigenous Pathogens 15
Knowledge 16
- International Information 17
- Canadian Information 17
- Regional Information - Atlantic 18
- Regional Information - Pacific 19

Knowledge Gaps 20
- Introductions and Transfers 20
- Chemicals Used to Control Health/Disease Problems 21
- Technological Constraints 21
- Diagnostic Sensitivity and Specificity Issues 22

Recommendations 22
Acknowledgements 23
References 23

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FOREWORD

Context

The Government of Canada is committed to ensuring the responsible and sustainable development of the aquaculture industry in Canada. The Minister of Fisheries and Oceans' announcement of the $75 M Program for Sustainable Aquaculture (PSA), in August 2000, is a clear expression of this commitment. The objective of the PSA is to support the sustainable development of the aquaculture sector, with a focus on enhancing public confidence in the sector and on improving the industry's global competitiveness. Ensuring the sector operates under environmentally sustainable conditions is a key federal role.

As the lead federal agency for aquaculture, Fisheries and Oceans Canada (DFO) is committed to well-informed and scientifically-based decisions pertaining to the aquaculture industry. DFO has an ongoing program of scientific research to improve its knowledge of the environmental effects of aquaculture. The department is also engaged with stakeholders, provinces and the industry in coordinating research and fostering partnerships. As a contribution to the Federal government's Program for Sustainable Aquaculture, DFO is conducting a scientific review of the potential environmental effects of aquaculture in marine and freshwater ecosystems.

Goal and Scope

Known as the State-of-Knowledge (SOK) Initiative, this scientific review provides the current status of scientific knowledge and recommends future research studies. The review covers marine finfish and shellfish, and freshwater finfish aquaculture. The review focuses primarily on scientific knowledge relevant to Canada. Scientific knowledge on potential environmental effects is addressed under three main themes: impacts of wastes (including nutrient and organic matter); chemicals used by the industry (including pesticides, drugs and antifoulants); and interactions between farmed and wild species (including disease transfer, and genetic and ecological interactions).

This review presents potential environmental effects of aquaculture as reported in the scientific literature. The environmental effects of aquaculture activities are site-specific and are influenced by environmental conditions and production characteristics at each farm site. While the review summarizes available scientific knowledge, it does not constitute a site-specific assessment of aquaculture operations. In addition, the review does not cover the effects of the environment on aquaculture production.

The papers target a scientific and well-informed audience, particularly individuals and organizations involved in the management of research on the environmental interactions of aquaculture. The papers are aimed at supporting decision-making on research priorities, information sharing, and interacting with various organizations on research priorities and possible research partnerships.

Each paper was written by or under the direction of DFO scientists and was peer-reviewed by three experts. The peer reviewers and DFO scientists help ensure that the papers are up-to-date at the time of publication. Recommendations on cost-effective, targeted research areas will be developed after publication of the full series of SOK review papers.

State-of-Knowledge Series

DFO plans to publish 12 review papers as part of the SOK Initiative, with each paper reviewing one aspect of the environmental effects of aquaculture. This Volume contains one paper: Disease interactions between wild and cultured shellfish.

Further Information

For further information on a paper, please contact the senior author. For further information on the SOK Initiative, please contact the following:

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AVANT-PROPOS

Contexte

Le gouvernement du Canada est déterminé à assurer le développement responsable et durable de l'industrie aquacole au Canada. Le Programme d'aquaculture durable (PAD) de 75 millions de dollars annoncé par le ministre des Pêches et des Océans en août 2000 traduit clairement cet engagement. Ce programme vise à soutenir le développement durable du secteur aquacole, surtout en améliorant la confiance du public envers l'industrie et la compétitivité globale de celle-ci. Veiller à ce que l'industrie fonctionne dans des conditions durables sur le plan environnemental constitue une responsabilité essentielle du gouvernement fédéral.

À titre d'organisme fédéral responsable de l'aquaculture, Pêches et Océans Canada (MPO) est déterminé à prendre des décisions éclairées qui reposent sur des données scientifiques éprouvées en ce qui concerne l'industrie aquacole. Le MPO mène un programme de recherches scientifiques pour améliorer ses connaissances sur les effets de l'aquaculture sur l'environnement. Le Ministère collabore également avec des intervenants, les provinces et l'industrie à la coordination des recherches et à l'établissement de partenariats. Le MPO contribue au Programme de l'aquaculture durable du gouvernement fédéral en passant en revue la littérature scientifique qui aborde les effets possibles de l'aquaculture sur les écosystèmes marins et d'eau douce.

Objectif et portée

Désignée projet sur l'état des connaissances, cette revue de la littérature définit l'état actuel des connaissances scientifiques sur les effets de l'élevage de poissons et de mollusques en mer et de la pisciculture en eau douce et fait des recommandations de recherches futures. La revue, qui se concentre surtout sur les connaissances scientifiques applicables au Canada, les aborde sous trois thèmes principaux : les impacts des déchets (éléments nutritifs et matière organique), les produits chimiques utilisés par l'industrie (pesticides, médicaments et agents antisalissures) et les interactions entre les espèces d'élevage et sauvages (transfert de maladies et interactions génétiques et écologiques).

Cette revue présente les effets environnementaux possibles de l'aquaculture documentés dans la littérature scientifique. Les effets environnementaux des activités aquacoles dépendent du site, des conditions environnementales et des caractéristiques de production de chaque établissement aquacole. L'examen résume les connaissances scientifiques disponibles mais ne constitue pas une évaluation des activités aquacoles spécifique au site. L'examen ne porte pas non plus sur les effets de l'environnement sur la production aquacole.

Les articles sont destinés à un auditoire de scientifiques et de personnes bien informées, notamment des personnes et des organisations participant à la gestion de la recherche sur les interactions environnementales de l'aquaculture. Les articles visent à soutenir la prise de décision sur les priorités de recherche, la mise en commun de l'information et les

interactions entre diverses organisations concernant les priorités de recherche et les partenariats de recherche possibles.

Rédigées par des scientifiques du MPO ou sous leur supervision, les articles ont été contrôlés par des pairs, ce qui assure qu'ils sont à jour au moment de leur publication. Après la publication de toute la série d'articles sur l'état des connaissances, des recommandations de recherches ciblées et rentables seront faites.

Série sur l'état des connaissances

Dans le cadre du projet de l'état des connaissances, le MPO prévoit publier douze articles de synthèse portant chacun sur un aspect des effets environnementaux de l'aquaculture. Le présent volume contient l'article suivant: Interactions pathogènes entre fruits de mer sauvages et d'élevage.

Renseignements supplémentaires

Pour de plus amples renseignements sur un article, veuillez communiquer avec son auteur principal. Pour de plus amples renseignements sur le projet de l'état des connaissances, veuillez communiquer avec:

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DISEASE INTERACTIONS BETWEEN WILD AND CULTURED SHELLFISH

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EXECUTIVE SUMMARY

This paper reviews the knowledge available on the wild-cultured host dynamics of shellfish infectious agents. As with finfish, shellfish health profiles are based mainly on knowledge derived from cultured stocks. This reflects an ease of access to cultured stock, which can introduce a sampling bias that complicates accurate pinpointing of disease sources.

Serious disease in shellfish caused by enzootic organisms generally arises from sub-optimal growing conditions, which render the animals more susceptible to opportunistic indigenous infectious agents. Alternatively, exposure of naïve and susceptible populations/species to 'exotic' infectious agents can also cause serious diseases. Differentiating opportunistic from 'exotic' infections is controversial when determining the aetiology of a 'new' disease. The emergence of an indigenous disease does not implicate accidental or deliberate introduction of animals from unscreened sources, as may be the case if an 'exotic' disease was detected. The evaluation of 'new' diseases depends on the ability to: i) identify the cause of the 'new' disease, especially because not all diseases are caused by pathogens; ii) develop or validate sensitive diagnostic techniques to accurately assess the distribution of the pathogen and ascertain if other hosts are involved; iii) trace the source (introductions, transfers, changing husbandry practices or changing environmental conditions, previously undetected 'background' infections); and iv) determine the relative significance of host physiology, genetic and ecological factors involved in the expression of the disease. Since shellfish culture is rarely practised in isolation from wild shellfish, the introduction of a new infectious agent into open-water shellfish culture can impact sympatric wild resources. Also, transplanted wild shellfish can be asymptomatic carriers of infectious agents that may infect cultured populations.

Opportunistic infections are most commonly documented in flow-through or semi-closed circulation facilities, where water exchange is limited, stocking densities are high and artificial feeding regimes are required. This provides the substrate for proliferation of ubiquitous aquatic microbes that would otherwise be benign (Elston 1984, 1989). The most frequently occurring opportunistic group are the Gram-negative Vibrionaceae bacteria (Walne 1958; Tubiash et al. 1965, 1970; Elston et al. 1981, 1982, 1987; Lodeiros et al. 1987; Dungan and Elston 1988; Dungan et al. 1989; Elston 1989, 1990; Nicolas et al. 1992). Sensitivity to Vibrio spp. varies among species and larvae are generally more susceptible than adult shellfish. Threshold tolerances vary and need to be established for individual holding systems, shellfish species and seasonal cycles of production (Sindermann 1988; Perkins 1993).

Most shellfish hatcheries use landfill sites to dispose of infected stocks rather than discharging them into the surrounding waters that supply the facility. Antibiotics may be applied, but the efficacy and expense of such treatments for ubiquitous opportunistic bacteria is questionable and has a direct and cumulative environmental impact (Plumb 1992). Uncontrolled antibiotic applications provide interim suppression, but not eradication, of losses and have led to rapid development of drug resistance in both pathogenic and non-pathogenic Gram-negative aquatic bacteria (OIE 1992; Plumb 1992; Subasinghe et al. 1995; Boyd 1999; FAO 1999).

KNOWLEDGE AND GAPS

There is little specific knowledge on the life cycle and ecology of most serious shellfish pathogens. In Canada, some effort has been directed towards understanding diseases of commercially exploited shellfish. The rapid development of shellfish aquaculture around the world, along with an increased demand for live shellfish, has escalated the need to prevent the spread of shellfish diseases. The risks associated with uncontrolled transfer and introduction of live aquatic organisms have long been recognised (Anon 1984; ICES 1988, 1995), especially for finfish species (FAO 1995; Humphrey 1995; Chillaud 1996; Humphrey et al. 1997; AQIS 1998; FAO/NACA 2000; OIE 2003a). In the last 20 years, the frequency of shellfish transfers has increased, due to the development of hatchery-based seed production, remote setting, and the increasing use of non-indigenous species in aquaculture (Kern 1994; Hine 1996; Minchin 1996, 1999; Bartley and Minchin 1996; Elston 1996).

Introductions and Transfers

- *Lack of baseline health data for local pathogens of 'new' species under culture, may impede accurate disease risk analysis, increase the difficulty of differentiating between exotic and endemic infections, and may hinder the identification of disease management options.*
- *Impacts on shellfish from accidental introduction of an exotic infectious agent could present consequences for both cultured and wild stocks under open-water culture conditions.*
- *Limited ability to detect sub-clinical carriers with subsequent development of more sensitive molecular tools may expedite turn-around time for diagnosis, but detection of other potential pathogens that may be significant to wild/cultured resources will not be possible, if detection is based solely on pathogen specific diagnostic tools.*
- *Lack of knowledge on the host ranges (i.e., all species susceptible to infection) for most shellfish pathogens seriously impedes the reliability of risk analysis results.*
- *Increasing dependence on remote processing and live-marketing facilities, usually not equipped with treated effluent or land-based waste disposal systems, complicates assessing the risk of inadvertent spread of infectious agents.*

Technological Constraints

- *Molecular tools to trace sources of infection are under development for only a few of the numerous shellfish pathogens of concern. These include probe production (Walker and Subasinghe 2000) for certain shrimp viruses (Lightner 1996b) and a few*

oyster pathogens (Stokes and Burreson 1995; Reece et al. 1997; Berthe et al. 1999; Berthe 2000; Carnegie et al. 2000; Russell et al. 2000). However, many of the procedures have not been fully validated (Cunningham 2002) and the interpretation of the results can be problematic (Bernoth 1999).

- *Focus on diagnostic assays for specific pathogens can preclude the detection of other pathogens not yet recognised because of knowledge gaps. This is mitigated through the use of histopathology, a non-specific screening technique.*
- *Cell-lines routinely used to isolate intracellular pathogens of vertebrates are currently lacking for both marine molluscs and crustaceans (Mothersill and Austin 2000). This has been a significant constraint to the detection and understanding the epidemiology of viral and other intracellular microbial infections.*
- *Difficulties in isolation of shellfish pathogens have also proven problematic for their culture and use in controlled infection experiments. These are necessary to examine Koch-Henle's postulates (cause-effect measures for disease) as well as accurately assess risk of establishment and disease spread (via carrier and normal hosts).*

Diagnostic Sensitivity and Specificity Issues

- *The development of more sensitive diagnostic techniques has focussed on pathogens that have a significant economic impact on production and trade. New pathogens, or those of more regional significance, rely on more 'traditional', but less sensitive diagnostic tests.*

RECOMMENDATIONS

- *Research is required to allow effective development of risk analysis procedures pertaining to shellfish diseases.*
- *Surveillance programs to assess the presence and prevalence of pathogens in wild and cultured shellfish in Canadian territorial waters are needed to protect Canadian aquatic resources from infectious diseases where early detection and intervention can significantly reduce impact and losses.*
- *Research is needed to improve diagnostic tools especially to enhance detection capability for significant sub-clinical microbial infections.*
- *Research is needed into pathogen life cycles. Information gained will be the basis of research into health management options and bolster risk analysis results.*
- *Research is needed on diseases of 'new' culture species, especially those with negligible or no health information.*
- *Assessment of factors associated with 'Introduction and Transfer' risks (especially fouling organisms and 'hitch-hikers') that may pose indirect health risks (e.g., as carrier reservoirs).*
- *Environmental suppression/exacerbation factors associated with disease expression are notably lacking in most shellfish health literature. The impact of disease under 'new' habitats or geographic conditions requires more detailed examination.*

INTERACTIONS PATHOGÈNES ENTRE FRUITS DE MER SAUVAGES ET D'ÉLEVAGE

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RÉSUMÉ

Dans ce document, nous passons en revue les connaissances sur la dynamique de la transmission d'agents infectieux entre fruits de mer sauvages et d'élevage. Comme c'est le cas pour les poissons, l'état de santé des fruits de mer est établi en fonction des connaissances acquises sur les stocks d'élevage en raison de la facilité d'accès de ces stocks. Cette façon de faire peut introduire un biais d'échantillonnage qui complique la détermination précise des sources de maladies.

Les maladies graves des fruits de mer causées par des organismes enzootiques découlent généralement de conditions de croissance sou-optimales, qui accroissent la susceptibilité des animaux aux agents infectieux indigènes opportunistes. L'exposition à des agents infectieux exotiques de populations ou espèces naïves et susceptibles peut également causer de graves maladies. Lorsqu'on détermine l'étiologie d'une nouvelle maladie, il est difficile de faire la distinction entre les infections opportunistes et les infections exotiques. L'apparition d'une maladie indigène n'implique pas l'introduction accidentelle ou délibérée d'animaux provenant de sources qui n'ont pas fait l'objet de dépistage, comme cela peut être le cas pour une maladie exotique. L'évaluation de nouvelles maladies repose sur les capacités à: i) déterminer la cause de la nouvelle maladie, en particulier parce que toutes les maladies ne sont causées par des agent pathogènes; ii) mettre au point et valider des techniques diagnostiques sensibles pour établir exactement la répartition de l'agent pathogène et déterminer s'il y a d'autres hôtes; iii) établir la source de la maladie (introductions, transferts, modification des pratiques d'élevage ou des conditions environnementales ou infections « de fond » non détectées auparavant); iv) déterminer l'importance relative de la physiologie de l'hôte et de facteurs génétiques ou écologiques dans l'expression de la maladie. Comme l'élevage de fruits de mer est rarement pratiqué isolément des populations sauvages, l'introduction d'un nouvel agent infectieux dans un établissement aquacole en eau libre peut toucher des ressources sauvages sympatriques. En outre, les fruits de mer sauvages transférés d'un lieu à un autre peuvent être des porteurs asymptomatiques d'agents infectieux pouvant attaquer des populations d'élevage.

Les infections opportunistes sont surtout mises en évidence dans des installations à système ouvert ou semi-fermé où l'échange d'eau est limité, les densités de charge sont fortes, et des régimes alimentaires artificiels sont nécessaires. Ces conditions sont propices à la prolifération de microbes aquatiques ubiquistes qui, parcontre, peuvent devenir bénins dans d'autres conditions (Elston 1984, 1989). Les bactéries gram-négatives de la famille des Vibrionacées constituent le groupe de microbes opportunistes le plus courant (Walne 1958; Tubiash et al. 1965, 1970; Elston et al. 1981, 1982, 1987;

Lodeiros et al. 1987; Dungan et Elston 1988; Dungan et al. 1989; Elston 1989, 1990; Nicolas et al. 1992). La sensibilité aux espèces de Vibrio varie selon les espèces de fruits de mer, et les larves sont généralement plus susceptibles que les adultes. Les seuils de tolérance varient et doivent être établis pour chaque installation d'élevage, selon l'espèce élevée et le cycle saisonnier de production (Sindermann 1988; Perkins 1993).

La plupart des établissements d'élevage de fruits de mer préfèrent éliminer leurs stocks infectés dans des sites d'enfouissement plutôt que de les rejeter dans le milieu aquatique. Les infections peuvent être traitées aux antibiotiques, mais on peut mettre en question l'efficacité et le coût de ces traitements lorsqu'ils visent des bactéries opportunistes ubiquistes, et ces traitements ont en outre des impacts environnementaux directs et cumulatifs (Plumb 1992). Les utilisations non contrôlées d'antibiotiques permettent de réduire temporairement les pertes, mais pas de les éradiquer, et elles ont entraîné rapidement le développement d'antibiorésistances chez les bactéries aquatiques gram-négatives pathogènes et non pathogènes (OIE 1992; Plumb 1992; Subasinghe et al. 1995; Boyd 1999; FAO 1999).

LACUNES DANS NOS CONNAISSANCES

On possède peu de connaissances précises sur le cycle vital et l'écologie de la plupart des agents pathogènes qui causent des maladies graves. Au Canada, certains travaux ont été effectués pour comprendre les maladies des fruits de mer exploités commercialement. L'expansion rapide de l'élevage de fruits de mer partout au monde et l'augmentation de la demande pour des fruits de mer vivants accroissent le besoin de prévenir la propagation des maladies qui touchent ces animaux. On connaît depuis longtemps les risques que posent l'introduction et le transfert d'organismes aquatiques vivants (Anon. 1984; ICES 1988, 1995) en particulier de poissons (FAO 1995; Humphrey 1995; Chillaud 1996; Humphrey et al. 1997; AQIS 1998; FAO/NACA 2000; OIE 2003a). La fréquence des transferts de mollusques augmente depuis 20 ans en raison de l'expansion de la production de naissain en écloserie, de l'établissement d'installations d'élevage dans des régions reculées et de l'utilisation accrue d'espèces non indigènes (Kern 1994; Hine 1996; Minchin 1996, 1999; Bartley et Minchin 1996; Elston 1996).

Introductions et Transferts

- *Le manque de données sanitaires de référence concernant les agents pathogènes locaux de nouvelles espèces d'élevage peut empêcher de bien analyser les risques de maladie, accroître la difficulté de distinguer entre les infections causées par des organismes exotiques et celles causées par des organismes endémiques et nuire à l'élaboration d'options en matière de gestion des maladies.*
- *Dans des conditions d'élevage en eau libre, l'introduction accidentelle d'un agent infectieux exotique peut avoir des répercussions tant sur les stocks d'élevage que sur les stocks sauvages.*
- *La mise au point éventuelle d'outils moléculaires sensibles offrira une capacité limitée de dépister les porteurs asymptomatiques et permettra d'accélérer le diagnostic, mais si le dépistage ne consiste qu'en l'utilisation d'outils diagnostiques spécifiques à un*

seul agent pathogène, il sera impossible de détecter d'autres pathogènes qui pourraient nuire aux ressources sauvages ou d'élevage.

- *Le manque de connaissances sur la gamme des hôtes (c.-à-d. toutes les espèces susceptibles d'être infectées) de la plupart des agents pathogènes des fruits de mer restreint considérablement la fiabilité des résultats d'analyse des risques.*
- *La multiplication des installations de transformation et de mise en marché de produits vivants dans des endroits reculés, lesquelles ne disposent habituellement pas de systèmes de traitement des effluents ou d'élimination des déchets à terre, complique l'évaluation des risques de propagation non intentionnelle d'agents infectieux.*

Contraintes Technologiques

- *La mise au point d'outils moléculaires pour déterminer les sources d'infection ne concerne actuellement que quelques-uns des nombreux agents pathogènes préoccupants. Ces travaux comprennent la production de sondes (Walker et Subasinghe 2000) pour certains virus de la crevette (Lightner 1996b) et agents pathogènes de l'huître (Stokes et Burreson 1995; Reece et al. 1997; Berthe et al. 1999; Berthe 2000; Carnegie et al. 2000; Russell et al. 2000). Toutefois, bon nombre des procédures élaborées n'ont pas été complètement validées (Cunningham 2002), et l'interprétation des résultats peut poser problème (Bernoth 1999).*
- *L'accent mis sur des essais diagnostiques visant des agents pathogènes précis peut empêcher le dépistage d'autres agents pathogènes pas encore connus en raison de lacunes dans nos connaissances. Ce problème peut être atténué par la réalisation d'études histopathologiques, qui constituent une technique de dépistage non spécifique.*
- *On ne dispose pas de lignées cellulaires permettant d'isoler des agents pathogènes intracellulaires de mollusques et crustacés marins, comme cela se fait couramment pour les vertébrés (Mothersill et Austin 2000). Cette lacune constitue une importante contrainte pour le dépistage d'infections intracellulaires microbiennes, virales ou autres, et pour la compréhension de leur épidémiologie.*
- *La difficulté à isoler les agents pathogènes des mollusques et des crustacés pose problème pour la culture et l'utilisation de ces mircobes dans des expériences d'infection contrôlée. Ces travaux sont nécessaires pour vérifier les postulats de Koch-Henle (critères de causalité d'une maladie) et pour évaluer avec exactitude les risques d'établissement et de propagation de la maladie (par les hôtes porteurs normaux).*

Questions Liées à la Sensibilité et à la Spécificité des Diagnostics

- *La mise au point de méthodes diagnostiques améliorées vise surtout les agents pathogènes qui ont d'importantes répercussions économiques. Les nouveaux agents pathogènes ou ceux qui n'ont qu'une importance régionale sont dépistés grâce à des tests diagnostics traditionnels moins sensibles.*

RECOMMANDATIONS

- *Il faut effectuer de la recherche pour permettre l'élaboration de bonnes procédures d'analyse des risques liés aux maladies des fruits de mer.*

- *Il faut mettre sur pied des programmes de surveillance pour évaluer la présence et la prévalence d'agents pathogènes chez les fruits de mer sauvages et d'élevage dans les eaux territoriales du Canada, afin de protéger nos ressources aquatiques contre les maladies infectieuses dont les répercussions (notamment les pertes subies par les aquaculteurs) peuvent être considérablement réduites par un dépistage et une intervention rapides.*
- *Il faut effectuer de la recherche pour améliorer les outils diagnostiques, en particulier ceux qui permettent de dépister d'importantes infections microbiennes asymptomatiques.*
- *Il faut effectuer de la recherche sur les cycles vitaux des agents pathogènes. Les connaissances ainsi acquises constitueront le fondement de la recherche sur les options de gestion sanitaire et accroîtront l'utilité des résultats des analyses des risques.*
- *Il faut effectuer de la recherche sur les maladies qui touchent les nouvelles espèces d'élevage, particulièrement celles qui sont méconnues sur le plan sanitaire.*
- *Il faut évaluer les facteurs de risque sanitaire indirect (p. ex., les hôtes réservoirs) liés aux introductions et aux transferts (en particulier les organismes accompagnateurs et ceux qui causent des salissures).*
- *Il faut étudier en détail les répercussions des maladies dans de nouveaux habitats ou de nouvelles conditions géographiques, car les facteurs environnementaux de suppression ou d'exacerbation des maladies sont très peu documentés dans la littérature sur la santé des fruits de mer.*

BACKGROUND

A fundamental biotic component of any ecosystem is the infectious flora and fauna associated with the host community. In all but sterile environments, some degree of infection is normal. Under an evolutionary adjustment process, the host and infectious agent establish a relatively steady-state relationship, where disease losses associated with infections are minimised and a reliable source of hosts for the infectious agent is maintained. Obviously, this is a precarious balance and any changes – natural or anthropogenic – can tip the balance against or in favour of the infectious agent. Many examples are well-documented under both terrestrial and finfish culture conditions, where the domestication process introduces varying degrees of physiological challenge that may suppress optimum immune defences (Kent and Fournie 1993). Parallel examples exist for shellfish, but the aquatic invertebrate immune system has only recently begun to be understood, so the actual 'balancing act' is not yet as well quantified as for vertebrate hosts (Sindermann 1990).

This paper tackles the knowledge available on infectious agents of shellfish that pertain to the dynamics between cultured and wild hosts. Non-infectious disease interactions concerning environmental biotic and abiotic pollutants are covered under the paper in this series by Cranford et al. (2003). Another important aspect of shellfish culture is the dissemination of epibenthic fouling organisms that use mollusc (and, to a lesser extent, crustacean) shells as substrata. This interaction is also discussed in the paper by Cranford et al. (2003).

Shellfish health profiles are based mainly on knowledge derived from cultured, rather than wild, stocks. This simply reflects ease of access to, and observation of, these compared to their wild conspecifics. This sampling bias tends to complicate accurate pinpointing of sources of disease problems, since after-the-event monitoring inevitably raises the "chicken and the egg" conundrum. Nucleic acid assays may help with this question in the future, by identifying the genetic characteristics of infectious strains (as was the case in tracking the origin of *Haplosporidium nelsoni* to Asia – see Burreson et al. (2000) and recent detection of this oyster pathogen in France – see Renault et al. (2000) – for details see below). Likewise, the continuum between wild and cultured stocks of bivalve molluscs, as well as many cultured crustaceans, makes a clear distinction between wild and cultured disease interactions difficult, if not impossible, to delineate.

Serious disease effects generally arise in shellfish (bivalves, crustaceans and echinoderms) from two scenarios:

i) sub-optimal growing conditions which render the animals more susceptible to opportunistic indigenous infections agents (Elston 1984);
ii) exposure of naïve and susceptible populations/species to 'exotic' infectious agents (Kern 2001; Harper 2002).

Differentiating opportunistic from 'exotic' infections is the first and usually the most controversial question addressed when attempting to determine the aetiology of a 'new' disease (i.e. a disease that had not previously been detected in a shellfish population). This

is also the pivotal question for determining what management strategies can be effectively put into place to minimise the effects on both the culture stock and contiguous wild populations. A complicating factor is that the occurrence of a disease is usually the result of a complex interaction between host, pathogen and their environment, and the presence/absence of a given pathogen may not correlate with disease occurrence.

'NEW' DISEASES

Addressing the appearance of a 'new' disease spans legal, managerial and scientific expertise, with varying degrees of involvement related to the severity of the immediate disease impact. Legally, the emergence of an indigenous disease (enzootic infections that are opportunistic or seasonally-driven) does not implicate accidental or deliberate introduction of animals from unscreened sources, as may be the case if an 'exotic' disease was detected. From a managerial perspective, greater emphasis can be placed on site and stock manipulation (husbandry practices) to enhance survival from an enzootic or opportunistic infection. This contrasts significantly with isolation, quarantine or eradication options required for control of an 'exotic' pathogen. Another managerial option, depending on the nature of the pathogen, may be selective breeding for resistant strains, a viable possibility especially since the advent of shellfish hatcheries. However, a selectively bred line may, over time, become more susceptible to other pathogens or less fit in other capacities due to the loss of heterozygosity.

Lastly, the scientific questions posed by a 'new' infection span:

i) identify the cause of the 'new' disease;
ii) develop and validate sensitive and preferably rapid diagnostic techniques to provide accurate assessment of the wild - cultured host infections and vectors of interaction;
iii) trace the source (introductions, transfers, changing husbandry practices or changing environmental conditions, previously undetected 'background' infections);
iv) determine the relative significance of host physiology, genetic and ecological factors involved in the expression of the disease.

Lack of information on some or all of these questions can complicate the risk assessment process. Unfortunately, the scientific aspects associated with a 'new' disease outbreak usually take years to resolve, with any degree of certainty. This frequently leaves managerial and legal questions, as well as accurate disease risk assessment, in limbo and may impede industry activities pertaining to cultured and wild shellfish productivity.

In the last 25 years, several new disease outbreaks have occurred in shellfish from various parts of the world including Canada. In an effort to control spread of significant diseases (regardless of origin), trade restrictions have been placed on Asian and South American shrimp, as well as European and North American bivalves. Many such restrictions follow guidelines outlined by the Office International des Épizooties (OIE – World Organisation for Animal Health, OIE 2003a; http://www.oie.int/eng/normes/en_acode.htm) in support of the World Trade Organisation Agreement on Sanitary and Phytosanitary Measures (http://www.wto.org/english/tratop_e/sps_e/sps_e.htm). Although such restrictions are not

generally undertaken with environmental protection as the prime concern, the result can be equivalent because prevention of spread of significant infectious agents reduces impacts on both wild and cultured resources. Once the scientific data on a new disease are collected, the importing authority may choose to continue the restrictions, lift them, or modify the import conditions accordingly.

Inadvertent introduction of an infectious agent into open-water shellfish culture can be assumed to equate to introduction and impact on sympatric (area of sympatry dependant on oceanographic or watershed characteristics) wild resources, since shellfish culture is rarely practised in isolation from wild shellfish. In addition, open water leases provide a plethora of reservoirs for all but the most fastidious or host-specific infectious agents. For example, nucleic acid (DNA and RNA) assays have clearly shown that many species of crustaceans can serve as asymptomatic carriers (or reservoirs) of infectious agents of some significant diseases of penaeid shrimp in Australia (Owens and McElnea 2000), India (Otta et al. 1999; Rajendran et al. 1999) and Thailand (Ruangsiri and Supamattaya 1999). In these cases, the cultured stocks were more susceptible than the wild crustaceans, indicating one or more of the following:

i) the susceptible stocks had compromised defence systems related to culture production (hatchery-produced larvae, holding conditions, inadequacy of feed, genetic selection, etc.);
ii) transmission of a pathogen, normally found in wild stocks, was favoured by the culture conditions (i.e. intense stocking densities that facilitate direct disease transmission); or
iii) the shrimp lacked prior exposure to a 'new' infectious agent, thus had no tolerance of, or immunity to, infection.

Alternatively, the wild stocks may be as vulnerable to infection as their cultured counterparts, but mortalities may go undetected due to rapid predation/decomposition. Also, wild stocks are often not assayed for pathogens.

OPPORTUNISTIC INFECTIONS

Opportunistic infections are most commonly documented in contained aquaculture facilities, where water exchange is limited, stocking densities are high and artificial feeding regimes are required. This provides the substrate for proliferation of ubiquitous aquatic microbes that, under other circumstances, would be relatively benign. By far the best-recognised group of opportunistic threats to shellfish health is the Gram-negative Vibrionaceae bacteria (Walne 1958; Tubiash et al. 1965, 1970; Elston et al. 1981, 1982, 1987; Lodeiros et al. 1987; Dungan and Elston 1988; Dungan et al. 1989; Elston 1989, 1990; Nicolas et al. 1992). The most common species are *Vibrio anguillarum*, *V. alginolyticus* and *V. tubiashii*, but most outbreaks are identified only to genus (Hada et al. 1984). Severity of infection is generally related to sub-optimal culture conditions (e.g. accumulation of dead or dying larvae, contaminated influent water, and/or sullied algal food) which enhance bacterial proliferation (Elston 1984, 1989). However, bacterial proliferation is not the only mechanism that dictates the pathogenicity of *Vibrio* spp. or

other opportunistic bacteria. In some cases, limited numbers of bacteria that produce toxic excretory products can have detrimental effects without overwhelming invasion of the host. Also, sensitivity to *Vibrio* spp. varies. Sindermann (1988) cites 10^2 *Vibrio* cells·ml^{-1} as potentially pathogenic to eastern oyster larvae (*Crassostrea virginica*), while other species, such as gold-lipped pearl oysters (*Pinctada maxima*) may tolerate 10^5 cells·ml^{-1} (Perkins 1993). Thus, threshold tolerances need to be established for individual holding systems, bivalve species and seasonal cycles of production.

Larvae are usually more susceptible to bacterial invasion than adult shellfish. *Vibrio* spp. produce exotoxins (ciliostatic factors and haemolysins) which cause tissue necrosis in bivalve larvae (Brown and Roland 1984; Nottage and Birkbeck 1986; Nottage et al. 1989). High water-temperature and excessive stocking densities increase the severity of infection (Elston 1989) especially in pre-metamorphic bivalves (although post-metamorphic juveniles may also be affected), hence the name 'larval necrosis' or 'bacillary necrosis' (Tubiash et al. 1965). *Vibrio anguillarum* isolated from the pericardial fluid of adult oysters with cardiac oedema was found to be highly pathogenic to experimentally infected eastern oyster larvae (Tubiash et al. 1970), however, pathogenic infections in adult bivalves are rare. Generally, *V. anguillarum* and *V. tubiashii* show minimal host-specificity and affect a wide range of larval molluscan species (Brown 1981; Jeffries 1982; Garland et al. 1983; Lodeiros et al. 1987).

Several species of *Vibrio* have also been implicated in diseases of abalone in culture facilities. In Tasmania, Australia disease outbreaks among cultured abalone (*Haliotis rubra, H. laevigata* and their hybrids) were associated with two species of *Vibrio* (*V. harveyi* and *V. splendidus* I) and *Flavobacterium*-like species. In most cases, stress factors (e.g. high temperatures, grading trauma, anaesthetics, gradual increase in salinity in the recirculation system, etc.) were reported to have precipitated the diseases (Handlinger et al. 2001, 2002). In Kanagawa Prefecture, Japan, *Vibrio carchariae* (possibly a junior synonym of *V. harveyi*) was isolated from cultured abalone (*Haliotis* (=*Sulculus*) *diversicolor supratexta*) experiencing a mass mortality epizootic (Nishimori et al. 1998). *Vibrio carchariae* was also identified as the probable cause of mass mortalities of *Haliotis tuberculata* in the natural environment along the Brittany and Normandy coasts of France and in a land-based abalone farm in Normandy (Nicolas et al. 2002). Dixon et al. (1991) reported that exposure to ozonated water and treatment (bath and injection) with a broad spectrum antibiotic (sulphadimidine sodium) was effective against bacterial infections (caused by *Clostridium lituseberense* or *V. alginolyticus*) in some abalone (*Haliotis midae*) in a South African experimental facility. However, Handlinger et al. (2002) found antibiotic use to give equivocal results on bacterial infections in Tasmanian farmed abalone.

High mortalities (up to 80%) of gold-lipped pearl oysters (*P. maxima*) in northwestern Australia have also been linked to *Vibrio* infections (Dybdahl and Pass 1985; Pass et al. 1987). *Vibrio harveyi* was isolated from the haemolymph of infected oysters and induced identical clinical signs in challenge infections of unaffected pearl oysters, including anomalous conchiolin deposits and disruption of nacre blister formation. The disease was linked to holding conditions during transportation from wild oyster beds to a pearl oyster

farm, especially when water temperatures were low (18° C). Mortalities were successfully reduced by avoiding transportation during the Austral winter, reducing stocking densities and shortening the shipment time (Pass et al. 1987). Thus, as with hatchery-induced *Vibrio* infections, this appeared to be a wild origin infection, exacerbated by the shellfish holding conditions.

One notable exception to the usual parameters of low pathogenicity in adult and open-water bivalves is *Vibrio tapetis*, the causative agent of 'Brown Ring Disease' (BRD) in Manila clams (*Venerupis* (*Ruditapes*) *philippinarum*) in Europe (Paillard and Maes 1994; Paillard et al. 1994; Borrego et al. 1996). *Vibrio tapetis* has caused mass mortalities of Manila clams along the Atlantic coast of France since 1987 and was the first *Vibrio* disease reported to affect adult bivalves growing in open-water (Maes and Paillard 1990). The bacteria attach to the periostracum causing abnormal thickening and a deposition of conchiolin along the edge of the shell. Manila clams were imported to Europe for culture purposes (Flassch and Leborgne 1992). The native clam species, the carpet clam (*Ruditapes decussatus*) can also be infected, but pathology tends to be less severe and usually is not fatal (Allam et al. 2001, 2002). This suggests that the Manila clam was either naïve to *V. tapetis*, or carried it, and European growing conditions induced pathogenic infections.

Another *Vibrio* species from Manila clams (VTP) is more pathogenic to larvae (Nicolas et al. 1992) than adults. As with *V. tapetis*, VTP shows unusual host-specificity, being non-pathogenic to other bivalves, such as *Pecten maximus* and *Crassostrea gigas*. In addition, VTP has a short survival time (4-5 days, compared with 7 days for *V. tapetis*) in seawater. Due to its appearance under hatchery, as opposed to open-water circumstances, VTP-disease was successfully eradicated by drying out the affected hatchery and surrounding facilities, and has not been reported since 1987 (Nicolas et al. 1992).

The only other example of an apparently species-specific *Vibrio* of bivalves is *Vibrio pectenicida* (Lambert et al. 1998). As with VTP, this is a hatchery-related larval problem for scallops (*P. maximus*), which shows little pathogenicity for larvae of Pacific oyster, clams or mussels. The bacteria appear to be ubiquitous in the marine environment, being isolated from the Bay of Brest, from which the hatchery water is derived. The levels of the bacteria were successfully suppressed via ultra-violet treatment of the influent waters.

Cytophaga-like bacteria (CBL), belonging to the gliding bacteria group (including *Flexibacter* spp.), cause hinge-ligament disease in juvenile oysters (*C. gigas, C. virginica, Ostrea edulis*) and clams (*Mercenaria mercenaria, Tapes philippinarum, Siliqua patula*) (Elston 1984; Dungan and Elston 1988; Dungan et al. 1989). Healthy bivalves appear capable of controlling the infection, whereas individuals under physiologically stressful culture conditions (as described for vibriosis above) appear more susceptible (Elston et al. 1982; Elston 1984). Infections generally affect juveniles <1 cm in length, causing liquefaction of the hinge-ligament (especially under warm conditions (10 – 20° C), and impeding feeding and respiration. *Cytophaga*-like bacteria are believed to be ubiquitous; thus a CBL disease outbreak in cultured stock is not likely to impact adjacent wild stocks of bivalves unless the latter are physiologically compromised in some fashion.

One final example of a proposed opportunistic infection is the etiological agent of Juvenile Oyster Disease (JOD) in *C. virginica* from the northeastern United States (Maine to New York). JOD causes sporadic high mortalities (up to 90%) in juvenile oysters (between 6 and 30 mm shell height) during the summer (Lewis et al. 1996). From experimental field studies, Barber et al. (1998) concluded that the occurrence of JOD was site specific (not dependent on source of seed), and that under challenge from JOD, selected oysters not only grew faster than unselected wild oysters, but exhibited an apparently gene-based tolerance of the disease. Although the cause of JOD remains elusive, recent studies suggest that a novel species of the α-proteobacteria *Roseobacter* group is involved (Boettcher et al. 1999, 2000). Interestingly, eastern oysters raised in JOD enzootic areas that were unaffected by JOD were colonized by *Stappia stellulata*-type α-proteobacteria (Boettcher et al. 2000). Considerable research is required to fully understand the relationship between shellfish and the microbes in the environment that they inhabit.

With respect to concern over 'environmental loading' due to aquaculture-related magnification of pathogen levels, most shellfish hatcheries and other culture facilities now use land-fill sites or equivalent disposal facilities for culled stocks, rather than dispose of such waste in surrounding waters that supply the facility.

Chemotherapeutants are generally restricted to systems where stocking densities, water volume / flow-rate and drug concentration can be controlled. For example, treatment of Brown Ring Disease using nitrofuran antibiotics was found to be effective under experimental conditions, but administration on a commercial scale was prohibited due to mutagen and carcinogen side-effects in vertebrates (Noël et al. 1990; 1992). Alderman et al. (1994) discussed treatment of Vibrio-P1 using flumequine (a group 4-quinolone) and noted questions with respect to accumulation in bottom sediments. Similar problems have been noted with the development of resistance to oxalinic acid (group 4-quinolone) and furazolidone by sediment bacteria (Nygaard et al. 1992; Samuelsen et al. 1992).

Commonly used prophylactic antibiotics in bivalve culture are penicillin-streptomycin or chloramphenicol combinations (Walne 1958; Bayne 1965; Minaur 1969; Elston 1990; Alderman 1992), however, these have been shown to be unreliable in seawater (Le Pennec and Prieur 1977). Although Chloramphenicol appeared promising for bacterial suppression in hatchery production of several bivalve species, concentrations that inhibited larval growth, or were toxic to the larvae, were inconsistent (Le Pennec and Prieur 1977). Chloramphenicol is now recognised as inducing rapid resistance in Gram negative bacteria, and its use in aquaculture is banned in several European countries, as well as Canada (Alderman et al. 1994). Cycloheximide, an antifungal agent (Ray 1965; Bower 1989) is toxic to the algal food of molluscs and ineffective against bacteria and non-metabolic stages of infective protistans. Resistant forms of the abalone parasite (*Labyrinthuloides haliotidis*) appeared within three treatments (Bower 1989). Since microbes in any hatchery comprise of an array of viruses, bacteria and fungi, use of a single antibiotic is intuitively ineffective and accelerates development of resistant of pathogens (Nygaard et al. 1992; Plumb 1992; Alderman et al. 1994).

Antibiotic applications have provided interim suppression, but not eradication, of losses and have led to rapid development of drug resistance in both pathogenic and non-pathogenic Gram-negative aquatic bacteria (OIE 1992; Plumb 1992; Subasinghe et al. 1995; Boyd 1999; FAO 1999). The pivotal paradigm shift, from 'quick fix' solutions, such as chemotherapeutant administration for opportunistic infections, to alternative husbandry methods, is a difficult one. The learning curve for shellfish production has mirrored that well-documented for finfish (Stoffregen et al. 1996).

In open-water culture systems, losses from opportunistic organisms are not well documented (especially for mussels and bottom-cultured oysters, clams and scallops). Nevertheless, direct intervention is not a normal shellfish culture method for disease control. Most control measures involve circumvention or rapid removal and land disposal of compromised stocks. A classic and extreme example of this is the action taken surrounding mass mortalities of Japanese pearl oysters in Mie Prefecture, Japan in 1997 (Miyazaki et al. 1999). Mountains of shell were dredged from affected beds to minimise the proliferation of mortalities. One of the problems associated with unselective removal of affected stocks is inevitable culling of potentially resistant individuals. Development of potentially resistant individuals has traditionally been linked to survivors of exotic pathogen infections (Ford 1988; Ford and Haskin 1988, 1995; Farley et al. 1996; Gaffney and Bushek 1996), however, for molluscs, there is no data to indicate this is equally applicable to chronic opportunistic infections.

One example of successful intervention in open-water is management techniques implemented to reduce losses from Denman Island Disease in Pacific oysters (*C. gigas*) on the west coast of Canada (Bower 1988). The disease is caused by an intracellular, microscopic protistan (*Mikrocytos mackini*) which also infects other oyster species (European oysters (*Ostrea edulis*), eastern oysters (*C. virginica*), and the indigenous Pacific Olympia oysters (*O. conchaphila*)) (Bower et al. 1994a). Actions that have successfully suppressed proliferation of the disease and reduced stock losses include:

i) harvesting of marketable oysters prior to the seasonal onset of the disease;
ii) holding sub-market, susceptible stocks at high tide levels on the oyster beds or in hanging culture to reduce exposure to water-borne infectious stages; and
iii) planting oysters on affected beds after June when disease transmission has ceased for the year.

In addition, studies on the lifecycle, host-pathogen interactions and epidemiology are ongoing (Hine et al. 2001). The parasite shows a distinct geographic distribution in the southern coastal waters of British Columbia (Bower et al. 1994b) and has recently been detected at two locations in relict populations of oysters in northern Washington State, suggesting a narrow environmental tolerance range for the infective stage of the parasite (United States Department of Agriculture, Veterinary Services, Center for Emerging Issues, http://www.aphis.usda.gov/vs/ceah/cei/mikrocytosis_usa0702.htm). Despite this, the parasite is subject to stringent surveillance controls with respect to live trade of *C. gigas* from Pacific Canada (OIE 2003a).

'EXOTIC' OR NON-INDIGENOUS PATHOGENS

On the international scene, there are several clear examples of the significant losses caused to both cultured and wild bivalve stocks by the accidental introduction of 'exotic' pathogens (e.g. Andrews 1980; Sindermann 1986; Bartley and Subasinghe 1996; Renault 1996). With respect to crustaceans, information on inadvertent disease introductions is almost exclusively limited to penaeid shrimp culture (Brock 1992; Lightner et al. 1992; Arthur 1995; Subasinghe et al. 1995; Lightner 1996a; Flegel and Alday-Sanz 1997). Details on the global movement of penaeid shrimp viruses and subsequent impact on production has been described in detail by Lightner et al. (1992) and Lightner (1996a). Likewise, crayfish plague (caused by the phycomycete fungus (*Aphanomyces astaci*) spread throughout Europe via restocking activity (rather than culture *sensu stricto*) is well-documented (Alderman 1996). For molluscs, an example of disease spread via transfer of stocks for culture purposes is bonamiosis of flat (= European, edible or Belon) oysters (*O. edulis*) throughout most of Europe. The disease is caused by a protistan haemocyte parasite (*Bonamia ostreae*) which was transferred with oyster seed from North America to France and Spain and subsequently throughout most of the Atlantic coast of Europe (van Banning 1982; Sindermann 1991; Cigarría & Elston 1997; Grizel 1997). This disease is of concern to Canada because *B. ostreae* is present in both Washington State (Elston et al. 1986) and Maine (Friedman and Perkins 1994) making it a threat to *O. edulis* cultured in British Columbia and Atlantic Canada.

Another protistan parasite, *Haplosporidium nelsoni* known as MSX (multinucleate sphere X), causes a serious disease of eastern oysters (*C. virginica*) along the east coast of the United States, and more recently within Bras d'Or Lakes, Cape Breton (S. McGladdery, DFO, Ottawa, Ontario and M. Stephenson, Gulf Fisheries Centre, Moncton, New Brunswick, unpublished data). The origin of this disease, first documented in 1957, remained a mystery until development of species-specific molecular diagnostic tools. Although a haplosporidian had been documented and described from *C. gigas* (Kern 1976; Friedman et al. 1991; Friedman 1996), the low prevalence of infection and a lack of associated pathogenicity in the Pacific oyster negated presumptive links to MSX disease of *C. virginica* prior to the availability of molecular evidence. Genetic analysis provided strong evidence that MSX originated from the western Pacific, possibly associated with the importation of *C. gigas* in the 1950s (Burreson et al. 2000). Another possible link to the western Pacific was the return of a large number of transport and naval vessels in the late 1940s and early 1950s to both coasts of the United States (US) (to California and to the Hudson and Delaware estuaries and lower Chesapeake Bay) where they remained moored for many years (Walt Canzonier, President, New Jersey Aquaculture Association, personal communication). Ballast and hull fouling have also been implicated in the recent spread of MSX disease into eastern Canadian oyster stocks. However, data are still being collected to assess this, as well as alternative mechanisms of spread from eastern United States. Direct introduction via Pacific foci of infection is deemed less likely than spread from eastern US MSX endemic waters to Atlantic Canada.

The recent development and use of molecular tools for the detection and diagnosis of shellfish pathogens will continue to have a significant impact on understanding shellfish

diseases. However, the routine use of DNA-based diagnostic techniques is hampered by a number of problems which may result in false positive or false negative results. Thus, efforts must be made to develop, validate and standardise rapid diagnostic techniques for diseases of concern (Berthe et al. 1999; Vasta 2001). Nevertheless, as molecular diagnostic techniques are developed for shellfish pathogens, important information on shellfish diseases will be revealed. For example, recent refinements were useful for distinguishing 'background' levels of SSO (*Haplosporidium costale*) infections from MSX (*H. nelsoni*) in neighbouring and overlapping oyster samples from Atlantic Canada. Without molecular analyses, distinguishing early, benign infections of both these species would not have been possible (Gagne and Stokes, personal communication). Also, the application of molecular techniques is proving vital in identifying and differentiating between viral diseases of penaeid shrimp (Lightner 1996b) and bivalves (Arzul et al. 2001). Such investigations were seriously curtailed in the past because of the lack of cell lines for marine shellfish.

Another notable example is Malpeque disease of Atlantic Canadian *C. virginica*. This was one of the first serious diseases to impact oysters. It devastated wild oyster beds in Malpeque Bay, Prince Edward Island (PEI), in 1917, following massive imports of seed from New England to replace over-fished wild oyster stocks (Needler and Logie 1947). Over 99% mortalities were reported, but the infectious nature of the disease was not recognised until the oyster stocks recovered from the initial outbreak, around the late 1920s. At this time, the fishery re-opened and the disease reappeared and spread throughout the rest of the Island, resulting in the second epizootic in the early 1930s. Subsequent outbreaks followed unapproved shipments of stocks to southeastern New Brunswick for processing. The resultant spread to the mainland led to a massive transplantation of oysters stocks throughout the southern Gulf to mitigate fishery losses with then-resistant PEI stocks (Drinnan and Medcof 1961). This also accelerated the spread of the disease. Investigations into the cause of this disease have spanned three generations of research scientists with the isolation of several candidate agents (Li et al. 1967, 1980). However, the actual candidate has not been detected using classic techniques. In 1997, an effort to evaluate the presence of the disease after over 30 years of clinical absence – using transplantation of historically naïve Cape Breton oysters revealed that the healthy southern Gulf of St. Lawrence oysters still carried sufficient infectious titres to elicit pathology in their naïve conspecifics within 18 months of exposure (McGladdery and Bower 1999). Molecular techniques are beginning to shed more light on this cryptic disease. Although the causative agent has not yet been identified, hopefully it will be revealed within this generation of scientific researchers.

KNOWLEDGE

Basic concepts and principles on the diseases of shellfish can be appropriated from the general knowledge that exists for diseases of vertebrates including humans. However, there is little specific knowledge on the life cycle and ecology of most serious shellfish pathogens. In Canada, some effort has been directed towards understanding diseases of commercially exploited shellfish. The following overview will reflect the generally

accepted concepts upheld by the international community and indicate what is known, as well as shortfalls in our knowledge base, on diseases of shellfish that occur in Canada.

International Information

The rapidly increasing development of shellfish aquaculture around the world, along with an expanding market demand for fresh (live) shellfish, has escalated the need for vigilance against the spread of shellfish diseases. The risks associated with uncontrolled transfer and introduction of live aquatic organisms have long been recognised (Anonymous 1984; ICES 1988, 1995; Elston 1996), especially for finfish species that are regularly transferred from one location to another (FAO 1995; Humphrey 1995; Chillaud 1996; Humphrey et al. 1997; AQIS 1998; FAO/NACA 2000; OIE 2003a). In the last 20 years, the frequency of shellfish transfers has also increased due, in part, to the development of hatchery-based seed production and remote setting, as well as to increasing use of non-indigenous species in aquaculture (Kern 1994; Bartley and Minchin 1996; Hine 1996; Minchin 1996, 1999). Concomitant with this increase in transfer has been the spread of significant shellfish diseases around the world (see above section on 'Exotic' or Non-Indigenous Pathogens).

Recognition of the correlation between shellfish transfers and disease spread has been reflected by global development of regulations or guidelines to control live imports of shellfish (mainly molluscs and shrimps) (Sindermann 1986; ICES 1988; Brock 1992; Carey 1992, 1996; Carlton 1992; Smith 1992; ICES 1995; AFFA 1999). Adherence to these guidelines is becoming more stringent as trade restrictions become increasingly applied under the World Trade Organisations Sanitary and Phytosanitary Measures Agreement (WTO-SPS Agreement), in accordance with standards and guidelines set out by the Office Internationale des Épizooties (OIE) Aquatic Animal Health Code and Diagnostic Manual (OIE 2003 a and b).

Canadian Information

As part of the process to revise the Fish Health Protection Regulations in Canada, Fisheries and Oceans Canada decided to broaden coverage beyond salmonids to include other finfish species and shellfish (including molluscs, echinoderms and crustaceans, see Bower and McGladdery 2000). It is anticipated that, although the Regulations may be shared between vertebrate and invertebrate groups, Manuals of Compliance outlining sampling protocols and diagnostic procedures will be separate, to take into account many fundamental differences in the culture of finfish and shellfish (Carey 1992, 1996). First and foremost is the recognised wild-cultured continuum for shellfish that is not so pervasive in finfish culture, where stocks are usually caged or grown in land-based ponds or production facilities.

An extensive list of shellfish diseases was compiled in 1994 (Bower et al. 1994b), in order to provide:

i) a strong scientific reference base upon which to justify the draft Regulations for shellfish;

ii) classification of disease agents according to a level of concern, and
iii) development of appropriate sampling protocols.

Drawing upon the primary literature and other information that is often not readily available (i.e. research laboratory reports, government technical reports and personal communications with colleagues), this comprehensive, world-wide synopsis on shellfish diseases of commercially important molluscs, echinoderms and crustaceans has been maintained and published as a web-site for ongoing reference purposes (http://www.pac.dfo-mpo.gc.ca/sci/shelldis/title_e.htm). This valuable synopsis was designed to be a source of basic information, including key references to pertinent literature that can be used to assist diagnosticians, and to provide scientific background for decision making by introductions and transfers authorities. It was also designed to be understandable by non-scientific readers, in order to assist growers with interpretation of the health information they receive from diagnostic laboratories and decisions received from licensing authorities, as well as to educate them to the risks inherent in production and shipment of live aquatic animals. The web-site was created to be as comprehensive as possible, by incorporating disease information about shellfish species both present in, and absent from, Canadian waters. It is not, however, the aim of the synopsis to review all of the details of each of the infectious agents. And, although most organisms (pathogenic and non-pathogenic) of commercially important shellfish species are presented, it does not include infections of shellfish of little or no current economic value (for which there is usually negligible information).

Regional Information – Atlantic

There are established health records for several molluscan species traditionally harvested and cultured on the east coast of Canada (mussels (*Mytilus edulis, M. trossulus*), eastern oysters (*C. virginica*), European oysters (*O. edulis*), hard-shell or quahaug clams (*M. mercenaria*) and soft-shell clams (*Mya arenaria*)). Notable exceptions are all species from Québec and Newfoundland waters. The latter provinces are beginning health screening of their principal shellfish (mainly mussels and giant sea scallops) to address this current lack of information. Results of this work in Newfoundland were recently published by Moret et al. (1999).

Most documentation on mollusc species in Atlantic Canada shows negligible differences in health profiles between wild and cultured species. This reflects the fact that brood- and seed-stock used for culture production are usually collected from wild stocks. These data are not surprising and are consistent with health profiles from wild and cultured molluscs elsewhere in the world. This means that any serious disease outbreak, such as MSX disease of eastern oysters caused by the haplosporidian protozoan parasite *H. nelsoni*, affects both wild and cultured oysters. Thus, any disease control measures have to be applied to the protection of both resource sectors (S. McGladdery, DFO, Ottawa, Ontario, unpublished observation).

Currently no crustaceans are commercially cultured in the Atlantic Region. However, lobsters are moved from capture sites and held in captivity for periods from hours to several months depending on marketing strategies. Although these holding conditions are

not considered as aquaculture, the associated translocation and pounding of lobsters could facilitate disease transfers because lobsters are known to host several opportunistic pathogens (e.g. *Aerococcus viridans* var *homari* (=*Gaffkya homari*, gaffkemia), Anophryoides (=*Mugardia*, =*Paranophrys*, =*Anophrys*) *haemophila* (ciliate or bumper car disease); see Loughlin et al. 1994 and Cawthorn 1997, respectively). However, this commercial activity has been conducted for about 50 years with no evidence of disease spread to wild stocks surrounding holding pounds or other live-holding facilities. Most diseases that appear in lobsters during live storage are associated with abrasion of the epicuticle (due to handling or over-crowding), leading to infections by opportunistic, chitinolytic, fungal or bacterial agents. The same situation is associated with stress-induced haemolymph infections, such as gaffkemia and bumper car disease. Furthermore, live-holding of lobsters provides opportunities to investigate diseases not readily observed in the field, and these studies have enhanced the understanding of natural population dynamics.

Studies including surveys of symbionts, parasites, pathogens and diseases of species new to aquaculture (giant sea scallops, surf clams, sea urchins, and sea cucumbers) have recently been initiated (e.g., Ball and McGladdery 2001). Thus, baseline data available for these species to differentiate opportunistic infections (relatively easily managed) from primary infectious pathogens (less easily managed in open-water culture systems), are currently lacking. This hinders the pin-pointing of problems and optimal disease control mechanisms during disease outbreaks (e.g. the Papatche Project on sea scallop mortalities being managed by the Québec Ministry of Agriculture, Fisheries and Food (MAPAQ) laboratory at Grande-Rivière). Also, it is difficult to predict how pathogens, known to occur in wild stocks, will impact cultured species. For example, it is not known if *Paramoeba invadens*, the cause of mass mortalities among wild green sea urchins, *Strongylocentrotus droebachiensis*, in waters off Nova Scotia (Jones 1985) will cause a problem when sea urchin culture becomes established. It is believed, however, that 'bald sea urchin' disease in captive sea urchins is related to opportunistic infections of the test (shell) following spine damage and loss through handling activities (Jones and Scheibling 1985; Roberts-Regan et al. 1988), rather than pathogen-specific bacteria.

Regional Information – Pacific

Historically, shellfish culture has been confined to introduced species (i.e. the Pacific oyster (*C. gigas*), Manila clam (*V. philippinarum*) and Japanese scallop (*Patinopecten yessoensis*)). Two diseases that are thought to have been introduced into British Columbia with the Pacific oyster (Denman Island Disease caused by the protistan *M. mackini* and nocardiosis, caused by the bacterial pathogen *Nocardia crassostreae*) have been clearly demonstrated to be pathogenic for other oyster species, under both field and laboratory conditions (Bower et al. 1994a). These may, therefore, have contributed to the current scarcity of the only indigenous species of oyster, the Olympia oyster (*O. conchaphila*). Manila clams have no apparent infectious diseases in British Columbia, however, highly virulent diseases, such as Brown Ring Disease (caused by *V. tapetis*) and perkinsosis (caused by *Perkinsus atlanticus*) have caused high mortalities in this and other species of clams in Europe and Asia. In contrast, all diseases detected in the Japanese scallop in

British Columbia, to date, seem to be indigenous with no apparent effects on other shellfish species, but with severe pathogenicity in naïve Japanese scallop stocks. The apparent lack of native diseases of Japanese scallops in British Columbia may be attributable to the introduction of this species for culture via the strict quarantine guidelines of the ICES Code of Practice (ICES 1988).

Recently, shellfish culture interests in British Columbia have been expanding to include several indigenous species, including the geoduck (*Panope abrupta*), abalone (*Haliotis kamtschatkana*), sea urchins (*Strongylocentrotus* spp.) and freshwater crayfish (*Procambrus* and *Pacifasticus* species). Very little is known about the diseases in these species. Thus, as for new species and new areas of shellfish culture in the Atlantic Region, baseline data are needed to help differentiate opportunistic infections (relatively easily managed) from primary infectious pathogens (less easily managed in open-water culture systems).

KNOWLEDGE GAPS

Introductions and Transfers

- Lack of baseline health data for local pathogens of 'new' species under culture impedes accurate disease risk analysis, differentiation between exotic and endemic infections, and identification of disease management options.
- Impacts on shellfish from accidental introduction of an exotic infectious agent can be assumed to present consequences for both cultured and surrounding wild resources under open-water culture conditions.
- Limited ability to detect sub-clinical carriers. Current development of more sensitive molecular tools may expedite diagnosis, but the pathogenic significance of sub-clinical infections to wild/cultured resources is difficult to assess without directed research on the putative pathogen. Any decisions based solely on molecular diagnostic results will be arbitrary until backed by knowledge on the transmission requirements of the pathogen.
- Application of sensitive and specific molecular diagnostic tools will expedite turn-around time for diagnosis, but other pathogens of significance to wild/cultured resources may go undetected, if health assessments are based solely on pathogen-specific diagnostic techniques.
- Lack of knowledge on the host specificity (i.e. all species susceptible to infection) for most shellfish pathogens impedes accurate risk assessment and mitigative options.
- Increasing dependence on remote processing and live-marketing of both wild and cultured shellfish complicates the risk assessment process. There is a significant gap in disease management because introduction and transfer controls stop at the harvest of aquatic animals. Thus, for shellfish, disease spread via processing and live markets are currently uncontrolled. (Note: assessment for human pathogens and toxins fall under *stringent* regulatory controls).

Chemicals Used to Control Health/Disease Problems in Shellfish Culture

- Chemicals are mostly limited to the removal of fouling organisms, including secondary sets of the same species under culture. This means that their application is stringently monitored in order to protect the health of the shellfish under culture or the surrounding resources that provide the seed and broodstock required for further culture. Hydrated lime baths (4% solution), saturated brine and heat (60°C) are the most commonly used treatments to control fouling on mussel lines and reduce starfish predation. Treatments are acute rather than chronic and aimed at times of year when problems exceed critical tolerance (MacNair and Smith 1999).
- Chemicals used for hatchery management of opportunistic larval bivalve pathogens are rarely used since it is usually more economical to discard contaminated seed batches and restart with new spawn than invest in chemical treatments.

Technological Constraints

- Focus on specific pathogens can preclude the detection of other pathogens not yet recognised because of knowledge gaps. This problem is currently mitigated by use of histopathology as a screening technique. Histological examination is non-pathogen specific and enables the detection of target as well as other diseases (where present in the tissue section and where they are of a size detectable at the light or electron microscope level of magnification). There is increasing development, however, of molecular-based detection technology, and the advantages these bring. As a consequence, specificity issues (i.e. capability of detecting other pathogens) may become more important.
- Molecular tools used to trace sources of infection; epizootiology and phylogeny are under development for only a few of the numerous shellfish pathogens of concern.
- Although molecular diagnostic tools and databases are still under development or entirely lacking for many shellfish pathogens, there has been a recent explosion in probe production for some aquatic pathogens (Walker & Subasinghe 2000; Cunningham 2002;) including certain shrimp viruses (Lightner 1996b) and a few oyster pathogens (Stokes and Burreson 1995; Reece et al. 1997; Berthe et al. 1999; Berthe 2000; Russell et al. 2000; Carnegie et al. 2000, 2003). Interpretation of results based solely on molecular diagnosis, however, requires some caution. Because the pathogen and pathology are not observed directly (McGladdery 2000), many of the procedures have not been fully validated (Cunningham 2002) and the interpretation of the results can be problematic (Bernoth 1999).
- Cell-lines routinely used to isolate intracellular pathogens of vertebrates are currently lacking for both molluscs and crustaceans (Mothersill and Austin 2000). This has been a significant constraint to understanding the epizootiology of viral and other intracellular microbial infections.
- Difficulties in isolating shellfish pathogens have also proven problematic for their culture and use in controlled infection experiments. These are necessary to examine Koch-Henle's postulates (cause-effect measures for disease) as well as accurately assess risk of establishment and disease spread (via carrier and normal hosts).

Diagnostic Sensitivity and Specificity Issues

- To date, development of improved (more sensitive) diagnostic techniques have focussed, almost exclusively, on pathogens that have a significant economic impact on production and trade. Such diseases provide the economic return required for the time and capital investment needed to develop molecular probes. New pathogens, or those of more regional significance, rely on more 'traditional' but less sensitive diagnostic tests.

RECOMMENDATIONS

- Research pertaining to the development of risk analysis procedures for diseases that affect aquatic animal health is required to make the decision process consistent, easier for managers and transparent to stakeholders. The need to apply risk analysis to diseases that affect aquatic organisms is widely acknowledged (Rodgers 2001). However, established risk assessment procedures for most aquatic animal diseases are still under development in Canada and other countries. Canada (Canadian Food Inspection Agency, and Fisheries and Oceans Canada) is a partner with FAO on a Hazard and Critical Control Point (HACCP) analysis of all seafood, from production to consumption – including human and aquatic animal disease (Roland Cormier, Canadian Food Inspection Agency, Fish, Seafood and Production - Program Network – Atlantic, personal communication) Other agencies are also involved in similar endeavours-(e.g.http://www.ecoport.org/default.htm).

- A comprehensive program that incorporates zonation and surveillance for pathogens in both wild and cultured shellfish is needed to protect Canadian aquatic resources from infectious diseases where early detection and intervention can significantly reduce impact and losses.

- Research is needed to improve diagnostic tools, especially to enhance detection capability for significant sub-clinical microbial infections. Current reliance on histology as the 'gold standard' is insufficient (Reddington 1995). (Note: Canadian Biotechnology Strategy "Field Validation Program" is currently addressing this question at DFO's Gulf Fisheries Centre and Pacific Biological Station for both finfish *and* shellfish).

- Research into the life cycles of shellfish pathogens that occur in Canada is needed to better understand the risks associated with the pathogens for both cultured and wild shellfish. The information gained during this research can be utilised in developing health management options to minimise negative effects of the disease.

- Research is needed on diseases of 'new' shellfish species coming into culture, especially those with negligible or no health information. This research is required to accurately distinguish 'opportunistic infections' - managed by husbandry manipulation - from 'primary pathogens' requiring more stringent management

and/or control mechanisms.

- Greater assessment is required for other factors associated with 'Introduction and Transfer' risks - especially fouling organisms and 'hitch-hikers'. (Note: Ballast water issues also translate into direct threats to both culture and wild stocks (Hayes and Hewitt 1998; Rigby et al. 1999)). Canada has initiated this process with the National Code on Introductions and Transfers of Aquatic Organisms (Anonymous 2002). However, current exclusion of processing and live-marketing sectors is being reviewed to determine how these should be included in overall risk assessment.

- Environmental suppression/exacerbation factors associated with disease expression are notably lacking in most shellfish health literature. Thus, the impact of disease under 'new' habitats or geographic conditions requires more detailed examination. For example, the risk of *Bonamia ostreae* establishment in European oysters (*O. edulis*) stocks cultured on both coasts of Canada is unknown given that this parasite currently occurs in locations with water temperatures warmer than those in Canada.

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